

Identification of Species in Populations

The analysis of population structure and dynamics is a complex task. It involves the identification of species within a population, the determination of their relative abundance, and the study of their interactions. This is often done through the use of statistical methods and mathematical models. The results of such analyses can be used to inform conservation efforts and to understand the underlying processes that govern the dynamics of populations.

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**Dynamics and Stability
of
Phyletically Packed Communities**

The results of the analysis of population structure and dynamics are presented in this report. The analysis was conducted using a variety of statistical methods and mathematical models. The results show that the populations are highly structured and that there are significant differences in the abundance of different species. These results have important implications for our understanding of the dynamics of populations and for conservation efforts.

September 12, 1979

Renewal Proposal, DOE Contract

DE-AC05-79ET01834

Departments of Zoology and Physics

University of Texas at Austin

R. H. Richardson and Jack S. Turner

with assistance of

Dan Vasco

1.2 Background

The idea of evolution of a species is the process of large numbers of small variations in genetic structure of a population over time. This process is often described as a "barrier" or "threshold" model. The results of such analyses can be used to inform conservation efforts and to understand the underlying processes that govern the dynamics of populations.

2.1.2 Identification Of Gamodemes In Populations -

The analysis of population structure has been hampered by the absence of tools to identify breeding units. Unless the members of populations were identifiable by striking visible or similar appearances, a subdivided local population would remain completely undetected. Workers generally were forced to assume homogeneity in natural populations even though detailed studies invariably indicated the presence of heterogeneity.

We have almost completed the reprogramming of analyses developed by Makela (1975) and Makela and Richardson (1977) to make them more easily used, better documented and probably more reliable. Since the data input format differs from earlier versions, we have delayed the completion of the analyses of the last year study in Hawaii until now. In addition, Dr. Peter Smouse, University of Michigan, and I have been developing a multivariate analysis of mixed gamodemes, which will be very useful in the complete analysis of such data (see Appendix C for draft of the manuscript). Therefore, the analysis of Hawaiian Drosophila, particularly D. mimica, is reaching a point where the results soon will be summarized for publication.

The results for 1974 and 1976 indicate that at least two gamodemes are identifiable in Bird Park, and their proportions change. In an earlier study a single homozygote of APH declined during a period of very acid rainfall during a volcanic eruption, but the original proportions of "genotypes" in the population returned quickly (Rockwood, 1969). We earlier postulated that the recovery might have reflected migration into the affected population from a population in a protected area receiving less rainfall (Richardson and Johnston, 1975), rather than selection against this particular electromorph. However, the most probable explanation is neither selection on this particular locus nor migration, but that the locus is a marker which distinguishes between two gamodemes. One gamodeme may have been more severely depressed by the adverse conditions during volcanic eruption, but recovered rapidly after the eruption ceased, or, alternatively, the fluctuation represented normal seasonal fluctuations of separate populations. Consistent with this latter interpretation is the recently published result of similar allozymic fluctuations in the absence of an eruption (Steiner, 1979).

2.2 Screwworms

The idea of eradication of a species by the release of large numbers of males sterilized by massive doses of X-rays was conceived about 25 years ago by Dr. E. F. Knipling and developed by him, Dr. R. C. Bushland, and their colleagues. The screwworm has been successfully eliminated from several Caribbean islands and from the southeastern United States. A "barrier zone" along the U.S.-Mexico border was established in 1964, with the intent of preventing a re-invasion of the United States each spring by the populations surviving below the freeze line. This barrier zone was effective for several years, but effectiveness in the last decade or so has been erratic.

In a recent symposium (Richardson 1978) we sketched a number of areas in which genetic and ecological processes could be factors contributing to the difficulties experienced by the "eradication" program. From our perspective outside the USDA program, it appeared that this massive effort in pest control had initiated a very interesting experiment from which we could learn many features about the responses of a community placed under intense stress. Our suspicions were that the pest was comprised of one or a few component species that would be differentially stressed, depending on their relationship to the population being released (Makela and Richardson, 1979). Indeed, we have discovered that this suspicion was substantially correct.

The results of the genetic analyses during the past year may be summarized briefly. There are many cryptic gamodemes (and presumably are different species or semi-species), many of which are sympatric. We have identified at least 7 such groups in the United States last year, 3 of which were found below the freeze line last winter in Mexico, and the others are yet to be found again this year. All of these were collected while up to 3000 sterile flies were being released per square mile per week, and control was limited to population suppression rather than eradication. The released flies were of a different type than any of those identified, however. While some of the types have been found only above the freeze line, their method of surviving freezing temperatures is unknown. Diapause or other adaptive responses not previously associated with the genus may be involved. We have found several additional species in Mexico which have not been found in the United States.

The genetic techniques used to distinguish the cryptic species involve chromosome karyotypes derived from quinacrine-stained metaphase chromosomes (developed and performed by Dr. John Ellison) followed by electrophoretic analyses of the same larvae from which the chromosome preparations are prepared. The collections of larvae typically are obtained from naturally infested wounds in livestock (collected by or under the guidance of Dr. W. W. Averhoff). The karyotypes occur in distinct groups, differing by heterochromatin concentrations (primarily on sex chromosomes) and chromosome morphology. We have not found types precisely the same as those with karyotypes published before the construction of the barrier zone. Thus, it appears that either these types have, indeed, been eradicated and now are extinct, or else they have been suppressed and/or their geographic range has been reduced such that our sampling has failed to reveal their presence. Concordant with the chromosome groups are sets of some allozyme electromorphs unique to some karyotypic groups, while there are strong differences in frequencies in other cases. In one instance a pair of sympatric groups are distinguishable only by allozyme assay.

Natural heterozygotes have not been found, suggesting natural hybrids are rare or non-existent. We have examined samples from more than 200 wounds, some of which represent a few matings. Egg clutches of different females may occur in a single wound. These cases are often observed when there are larvae of different instars of development. Many of these taxa are sympatric, and collections may be made of several types in the same area, sometimes from the same wound.

We have found examples suggesting strong differences in host preferences, even between such similar hosts as cattle and sheep. Niche separation in this way closely parallels the plant substrate preferences in Drosophila we have studied previously. Also similar to Drosophila we have studied, there is accumulating evidence that some species oviposit less than a dozen eggs in a clutch in some individual hosts, while others are more likely to deposit up to about 50 eggs, and a few typically deposit up to 200 eggs. In the latter case and in some of the intermediate cases they also will re-oviposit on previously infested wounds, whereas in some cases there is only one oviposition per wound. Clearly these species represent a wide range of reproductive strategies, from "r-selected" to "K-selected" species.

As will be addressed in the section below, this extreme diversity of reproductive strategies between closely related, sympatric species is intriguing. As in Drosophila, such diversity of screwworms is very difficult to discover by casual observation of individuals. In Drosophila we commonly discover the existence of some of the cryptic species when we attempt to culture certain types. Since the K-selected species often have specialized cues for oviposition, and often lay only a few eggs, culturing in the laboratory is a challenge. When we have a "single species" which is easily cultured, but then find certain lines are very difficult to culture, we have learned to take this anomaly as a strong clue of the presence of a cryptic K-selected sibling species. Such anomalies will likely be important in future studies, whether they involve Drosophila, screwworms, or other taxa.

The geographic ranges of the cryptic species of screwworms also are highly variable. Some extend over a thousand miles. One type is found from Mazatlan to Phoenix, while another sometimes extends between San Antonio and El Paso and south from San Angelo across "west Texas," presumably into the Chihuahuan Desert. Still others have only been found in a single locality, such as north of Tampico within 175 miles of the Gulf of Mexico and with a north-south range of about 200 miles. A few have only been found in a single collection, which may suggest either a restricted range, that or they occur in very low frequencies, causing difficulty in sampling.

Although only historical speculations may be made at the present time, it is plausible that the initial successes of the eradication program represented the extreme suppression, or possible extinction, of a dominant species in Florida and Texas, with subsequent outbreaks representing the increase of previously secondary species. As the sterile males to be released in the future are matched to specific species, we will be able to observe changes in the relative proportions of the remaining species. Thereby "species releases" (significant and permanent expansion in numbers or the range of a natural population) for secondary species will be monitored. Also, it is likely that we will relate some of these census and geographic changes of the species to weather and other variations occurring over a few years. These factors may demonstrate some characteristics besides competition or interference by related species which have restricted the species ranges or retarded their seasonal increase.

Although this contract provided part of the support for this work last year, this will not be the case in the renewal period. We presently are continuing some of the genetic and systematic surveys associated with pest control with the financial support of the USDA and the livestock industry. Although this work is focused on the control or eradication of a pest (which we found to be a complex of species), we will continue to accumulate data of more basic interest.

With the theoretical studies proposed for this contract in the next period, we hope to gain some insight into the community dynamics ("species release" of secondary species, as various numerically dominant ones are eliminated or suppressed, for example) and develop appropriate models for the type of responses precipitated by an autocidal biological control program or any other selective reduction in the Malthusian parameter for the intrinsic rate of population increase. In addition, responses of other members of a community and the resulting effects on stability of the community will be brought into focus by the models.

2.3 Ticks

The research on ticks has been supported by the USDA during the past year (while this Contract with DOE has supported the research on Drosophila and screwworms). However, for completeness of the unpublished background data as it relates to our plans for the next contract period with DOE, a brief review of this work will be given.

Ticks represent a different type of experimental material to either screwworms or Hawaiian Drosophila, having received a concerted effort over the past several decades to reduce the population by chemical insecticides. In some cases, eradication of particular species by insecticide treatments over large areas has been attempted. (They are vectors for a number of diseases of livestock and humans.) Our work has centered on those which have received persistent treatments of various insecticides for a number of years. In certain cases species have managed to extend their ranges greatly in the face of such stresses as the insecticide treatments. Clearly the community has been altered, likely by human activities, but not necessarily to the detriment of the pest species. We are now interested in identifying the reproductively isolated populations (gamodemes, which in many instances probably represent cryptic species). From this base line we hope to gain some insights relating the range extensions to the factors responsible for range extensions, whether they be reflections of genetic changes in the population, or other factors having a closer connection to environmental modifications by man. The understanding of community instability, whether reflecting changes involving local or absolute extinction, or greater packing of a local community by the range extension into a new area, represents the broad objective of this research.

In collaboration with USDA researchers (coordinated by LaWanda Hunt) we have identified several cases of presumptive cryptic species in three presently recognized species of ticks. We are now in the process of identifying the ranges of each gamodeme, as well as conducting various breeding tests among the several populations we have found.



THE UNIVERSITY OF TEXAS AT AUSTIN

AUSTIN, TEXAS 78712

Department of Zoology

October 10, 1979

Dr. Ray Gagne
USDA/SEA/AR
Beltsville Agricultural Research Center
Beltsville, Md. 20705

Dear Ray:

I just received from Dr. Snow a day or two ago a copy of your report of September 18 to Dr. Lindquist. Of course, you had discussed these interpretations with me earlier, so I was not surprised with that, but I am disappointed that we didn't get the chance to go over together the preparations we have made. I don't think that all the things we are impressed with were included in your evaluation. Also, you may have received a copy, but just in case you didn't, I'll send you our quarterly report telling of the problems of contamination in the stocks at Mission. We have not had a chance to evaluate the situation for Tuxtla. As John and I mentioned to you during our brief visit at the airport in McAllen some time ago, such problems would seriously interfere with your evaluation.

I hope you will consider the confusion unreliable material could cause, and will reconsider what I take to be your present position, namely, that there is not much in the screwworm types we have identified to demand further attention from you. We feel very strongly that your help is needed, and I hope you will continue actively working on these types using new specimens.

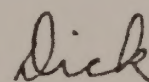
In your report there are several points we could discuss, but I think they largely are irrelevant to the crux of the things we have found so far that apply directly to the screwworm eradication program using sterile male release tactics. Many definitions of species, of course, are used in different contexts, and the semantic problems are gargantuan. Nevertheless, there is very strong evidence indicating little or no hybridization between several of these types under natural conditions. Whether one labels them different species, or, as we prefer, gamodemes, is tangential to the eradication efforts. We need morphological features to more easily separate members of two sympatric non-interbreeding populations in order to better evaluate their significance to the program, under un-natural conditions of sterile fly release. Your experience and access to notes and collections place you in a unique position to contribute to this objective.

If it is at all possible, I would like for you to spend some time in Austin, where we could compare notes and interpretations, and come to some agreement on the significance of the differences we have seen in the terminalia. Wil Averhoff will have new material taken from offspring grown from wild females that have been individually egged in Tuxtla,

thereby minimizing the chances of contamination. Although these are collections from Arriaga, they likely will include some of the types similar to those we have seen both in Aldama and in western Mexico. By the time you would arrive, we would have the chromosome and protein data to categorize them according to reproductively isolated populations.

If you are able to attend the next technical meeting in Mission, as Dr. Snow suggested, this would be an ideal time to include in your itinerary a stop-over in Austin. Although John and I are tied down with a teaching schedule, we would have plenty of time to spend on the problem with you. The best time might be the weekend before the technical meeting, if you could fly in on Friday night or Saturday morning before the meeting, which usually is on Tuesday.

Sincerely,



R. H. Richardson
Professor

cc: J. W. Snow
B. Hightower
R. W. Hodges
L. V. Knutson
L. LaChance
D. A. Lindquist
M. E. Meadows
N. L. Meyer
B. Miller
Mission & Tuxtla Researchers
J. Novy
N. V. Pineda
E. A. Taylor
M. Vargas
L. E. Wendel
D. D. Wilson

UNITED STATES DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION ADMINISTRATION

AGRICULTURAL RESEARCH
SOUTHERN REGION
Screwworm Research
P. O. Box 986
Mission, Texas 78572

October 2, 1979

Subject: Screwworm Morphology

To: Ray Gagne
Research Entomologist
USDA, SEA, AR
Northeastern Region
Beltsville Agricultural Research
Center
Beltsville, Maryland 20705

I received a copy of your Sept. 18 Memoranda to Dr. Lindquist and found it extremely valuable and interesting. The information is of such significance that I have taken the liberty to circulate it widely. I hope you still plan on attending the next Quarterly Meeting in Mission and make a presentation of your data and observations. The presentation should improve our understanding of the entire genus and help us make better program decisions.

J. Wendell Snow
J. Wendell Snow
Research Leader

cc: B. Hightower, APHIS-Mexico City
R. W. Hodges, SEA-Beltsville
L. V. Knutson, SEA-Beltsville
L. LaChance, SEA-Fargo
D. A. Lindquist, SEA-Beltsville
M. E. Meadows, APHIS-Mexico City
N. L. Meyer, APHIS-Washington, D. C.
B. Miller, APHIS-Mission
Mission & Tuxtla Researchers
J. Novy, APHIS-Mission
N. V. Pineda, APHIS-Mexico City
~~R. Richardson, Univ. of Texas-Austin~~
E. A. Taylor, SEA-Weslaco
M. Vargas, APHIS-Mexico City
L. E. Wendel, APHIS-Mission
D. D. Wilson, APHIS-Mission

UNITED STATES DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION ADMINISTRATION

AGRICULTURAL RESEARCH
NORTHEASTERN REGION
BELTSVILLE AGRICULTURAL RESEARCH CENTER
BELTSVILLE, MARYLAND 20705

RECEIVED SEP 24 1979

September 18, 1979

SUBJECT: Interpretations Following a Study of the External Anatomy
of the Primary Screwworm

TO: D. A. Lindquist

THROUGH: R. W. Hodges *RWH*
L. V. Knutson *LK*

Since my July 9-12 trip to the Screwworm Laboratory in Mission, Texas, when I became involved with the question of screwworm "biotypes," I have spent most of my research time studying specimens of the primary screwworm, Cochliomyia hominivorax, and the other 3 species in the genus. My analysis of the body characters, particularly the reproductive organs which are among the most diagnostic species characters of insects, leads me to the conclusions that the screwworm fly is remarkably homogeneous and that the differences found in the chromosomes and proteins are apparently not reflected in external anatomy.

I have seen specimens of C. hominivorax from many of the Mexican localities at issue: Sonora, Vera Cruz, and various lines from Aldama. In addition, the National Collection has 400 specimens from many separate collections made in most South American countries from as far south as Uruguay and central Chile, from the West Indies and Central America, and from the United States when the screwworm occurred as far north as Iowa and Virginia. I can see no differences between male specimens of C. hominivorax from Chile and those from Texas!

The National Collection has also adequate material for study of the other 3 species in the genus, especially of C. macellaria, the other species that covers the entire range of C. hominivorax and more. Compared to one another, each of these 4 species can be seen to be distinct and easily differentiated by external anatomy. But within each species, there is very little variation, and that variation is of a kind that can be seen between the right and left sides of a specimen. I have seen no "hybrids" between 2 of these 4 species. In fact, experiments at the Mission, Texas, lab have shown that mating between the primary and secondary screwworm is mechanically unfeasible because of the "lock and key" arrangements of the genital organs.

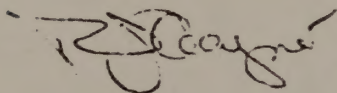
The presumption of R. Richardson et al. of the University of Texas that C. hominivorax is a complex of species is based, first, on an analogy with fruit flies on which Richardson et al. have had experience as geneticists, and, second, on alleged differences in the sclerotized structures of the

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male reproductive organs among specimens from northern Mexico. The analogy with fruit flies is tenuous. In only a few cases have chromosomal differences not been reflected in external anatomical differences, and, in those, the species have restricted ranges. Anyway, all fruit fly experts do not agree on the results, some thinking that if 2 "species" are not separable, they are so closely related that it is no help to consider them separate species.

Differences in male genitalia among specimens as presented by Richardson are artifacts due to differences in the length of time specimens have been in clearing and staining solutions and in the difference in angles from which the specimens have been photographed. The sclerotized parts of the genital structure are a system of levers interconnected by a complex of muscles that together act to grasp the female postabdomen, clear a passage for, insert and fasten the penis in close juxtaposition to the female sperm reservoir openings. The claim that, say, one structure, the epiphallus, is bent forward in specimens of one population and backwards in another is meaningless because the muscles in these specimens have been dissolved so that the epiphallus is flapping around loosely. The basiphallus works one way during mating, to prevent the penis from being inserted too far into the female for proper placement. Any change that developed in a male structure would have to be reflected in a corresponding female structure because of the "lock and key" arrangement of the genital organs.

The differences in karyotypes and proteins found in C. hominivorax probably only reflect individual or populational differences. I have seen nothing in the external anatomy of these flies to support the contention that what is now considered the primary screwworm is a complex of several species.



Raymond J. Gagne
Research Entomologist

Conference between USDA and UT Representatives

Concerning Proposed Mexico Field Test

Robstown, Texas - Jan. 11, 1980

Recorded Conversation

Tape 1 - Side 1

Richardson: ... the main survey ... chromosome types ... is beginning to compare enzyme patterns with chromosome groups and in some cases possible further subdivision in chromosome groups might be made with more subtle differences. If the enzyme data also suggest differences in gene frequencies within some of these subgroups, then we want to take a closer look at it (them) but basically a number of these are in culture at Tuxtla and that sorta sets the stage to go into the next area of work now that we've got them and we sorta know what's there - what do you do with them? That's why I wanted to get some discussion on it while Will was still up here and he can participate. The general plans coming up would be to now move as rapidly as we can with some reasonable questions into a field test in Arriaga. There are a lot of questions that one can ask and one of the things that I'm concerned about right now is getting a broader base of input, is choice of priorities on what questions we want to ask out of that test, and in fact there may be a need for more than one test -- that's one set of possibilities that's to be determined. In the process of working down there after having looked over the surveys from Sonora, Sinaloa (the West Coast) and from Aldama (the East Coast) with first year's survey results from north of the border and a smattering of samples as represented from Vera Cruz to Aricruz (some of the material is still in culture), I think we have a very general idea of the diversity of screwworms in Mexico but its a very general idea. Talking about Central Mexico, it's unsampled, totally unsampled. Southern Mexico from essentially Aldama South on the East Coast and with the small sampling in Sinaloa but from what we do have in Sinaloa (what everybody has from Sinaloa) and Arriaga as a whole, ... so when those are further sampled I think we can say we have a good idea of what we have in

Mexico but so far we've got more or less half the country surveyed with some idea of the diversity and some of the types are fairly wide spread, essentially making a "V" in Mexico, on the East Coast, Arriaga, West Coast and that's where our samples are. They may also be in the Central part if we can get samples from there. Other types are very localized, found either only in Aldama, or only in Arriaga -- this type of thing. And, in part I think as we discuss the kinds of questions we might want to ask in the Arriaga tests, it'll depend on our guesses based on this survey data that is available, even though it's limited, it will give us some ideas I think about how to set up the Arriaga test. In this thing here on field tests I just raised a number of the questions which we could very quickly get into. For example, do we want to concentrate on the release of the type that is generally widespread over Mexico, or do we want to work with an endemic type? I would guess that we would probably want to work with the general type and there are various reasons for that. The survey data sorta gives us a basis of which then we can try to design some sort of testing protocol in Arriaga that gives us some basis to generalize back into other areas of Mexico besides Arriaga and how good a generalization that is will be limited primarily by the survey data that we have elsewhere in Mexico, etc. I think that may give you some idea of what the bigger picture is, but getting down to the details of what are we gonna do and in terms of which strains to focus on and what are we going to do in terms of how are we going to focus on them, as far as I'm concerned is still very much up in the air. And I don't think we can answer that question today, but I think we can sorta lay out some of the ideas and begin to think about the various aspects of it -- we can do really two things. One is, this will generate new ideas which we need to be continuing to discuss as the time for the field test itself approaches and also it may give us a chance to find out where we have holes in the information

available so that we can begin to focus on filling those holes and not get caught at the last minute wishing we'd had a piece of data, you know, to make a decision, and not have it and just have to toss a coin, and go one way or the other. Is that more or less what you were looking for?

Coppedge: Yes, I guess that's a good point -- which obviously keeps it open. I've tried to talk to everybody in our organization that's interested, who might be involved in the test, and I think it's pretty well unanimous everyone feels that it's very important to get this thing in the field this year in Arriaga.

Richardson: I agree.

Coppedge: I hope, and just being honest, a lot of other things that I see here, ecological studies and things like this, it would be nice if we could do them, but I think they have got to go down on the list of priorities.

Richardson: There is a lot of that you do at the same time and it's not an "either or" -- there are a few places that's an "either or" and at those places we have to try to set up the tests so that if we decide later to go the next step that it comes -- first of all, we can eliminate possibilities -- we can't actually burn bridges if we aren't careful and if we think there is a possibility later on that we would want to ask one of the other questions, then we want to be careful not to burn a bridge. The other is we may be able to, with no additional effort, get information which would be preliminary but nevertheless be important, if we go another cycle into some of the other questions, so that we have some information, some feel, on how to do it efficiently and effectively. I think the field test certainly is a very high priority item and we're gonna have to design it in part wishing we had more

information -- I think that's a given, but there's no reason that we have to go blindly and we may be able to supply ourselves information right now, you know, of course we're not quite ready to get there. In the meantime, we can still get some more.

Coppedge: I think it's reasonable in most people's thinking -- it's great to know the different types in Mexico. The real question is what is the significance of this to normal application?

Richardson: That's not a simple question -- that's why I said a while ago that when we do a field test we presumably want to be able to generalize as best we can back to the rest of Mexico, and not just learn something about Arriaga and how you set the field test up -- you may be able to learn a lot about Arriaga and still not know anything about how it relates to the screw-worm eradication in the rest of Mexico or if you set it up you will have some basis to extrapolate elsewhere. That's why I said a minute ago I presume we would want to focus on something down there that's also found elsewhere in Mexico -- that immediately seems to be a reasonable basis for extrapolation. At the same time you do it down there we may also be able to find out some things that are, strictly speaking, relevant only to Arriaga but still would give us some other indicators of hazards down the road if indeed there are such. Let me give you an example of (I'm reading into what you were just saying about the ecological stuff) -- I'm reading one of the things that might fit in that category is the idea of the species release which is strictly an ecological concept and it may or may not be relevant to screwworms -- we simply have no way of predicting, but suppose, let me play a scenario for you -- to show you how we might want to keep that possible problem in mind so that if we set the test up and then monitor it properly we may be able to say

something about that particular problem. But suppose you pick a common kind of screwworm in Arriaga that you find representative of Sinaloa and/or Sonora, and something similar to it as best we can tell on the East Coast, and you release those sterile flies and monitor (taking that type out); if you have other types down there that are resistant to being taken out (that is, it may be possible to take them out but you may have to load them much heavier with the mis-match) and we then come to the situation of do we want to go ahead and load them as heavy as necessary to take them out, or do we want to back off -- stop at that point and leave them there. And by that time maybe having taken out the type that's a match -- then stop the release -- you've eradicated one -- you've not eradicated someother. That, to an ecologist says there's now an open niche. The type that's gone leaves an open niche. The next question would be what happens to some of those that remain. How do they react to that open niche? One of the ones that was in low frequency, maybe not economically important as a pest, at that point may break out. That would be a species release and fill that niche. Now that niche would have been the niche of the one that was common before. Now then, an uncommon kind would become a common kind --sometimes there's a lag period in there. This is a situation analogous to what happens when you get a host shift, say in a plant pest. It gets introduced into the area, there's no host match, there's a lag time there and after a while it shifts hosts and then you get pests which develop de novo. Now, why would we want to know that among screwworms when we can take them all out in Arriaga (supposing we could -- if you go ahead and load it heavy enough and eradicate every type of screwworm there -- why do we still want to have any information about the possibility of a species release)? Well, this goes back to the nature of our survey. We're able to detect only screwworms (types of screwworms) that are relatively common --

even the most uncommon kind we've got, looking at the sampling pattern that we have, we are still likely to miss still more uncommon kinds of screwworms. And, since we don't know the total diversity -- we only know sorta the more common of the types that are in Mexico one would presume (if an eradication program goes through) and because there typically is a lag period for species release what one might have would be the country declared eradicated screwworm-free -- stop the release -- and two years, three years later, all of a sudden you get a new outbreak and it might be then called an introduction which it might be, or it might be an uncommon kind that you didn't know was there that has become more common and becomes a new pest as a species release. So, if we had some information as to, for example the kind of lag period that might happen in an Arriaga test, this might give us some idea of how long monitoring facilities and, you know, watchdogging after eradication was declared complete, how long one might really have to concentrate on that. It would be an extrapolation because we are not talking about the same kinds that presumably would be studied in Arriaga that we already know about. That's the type of foundation I think that we may be able to get some information on if we decided to go to the trouble -- we have an opportunity at least to get some information. If we exclude that opportunity from our testing protocol then we should exclude it knowingly and decide explicitly that this is not something we wish to raise a question at this time as being important enough to be worth our trouble.

Coppedge: I think many of us understand that ... two things that I'm interested in now We need to be able to do some planning for allocation of people and resources and too, talking to Mr. Taylor, about additional funding, he told me as soon as we could bring a general plan of field work from which we could

develop a time table so that we could see all the problems so we'd have more ammunition to go to ... then, I think we'd be in a better position We just couldn't go off and request additional funding without it.

Richardson: Of course, that's not surprising. That's what we were talking about the other day -- today and yesterday might have been best reversed. Okay, that's reasonable I think.

Coppedge: I'd like to see us somehow or the other -- get this field thing up and get some sort of plan together -- sketch this time table -- maybe we won't do it today.

Richardson: I hope we can move in that direction, if not actually sketch a tentative one in. I don't know how far we'll go today on it. I think in the discussions that John and Will and I have had, it's clear that it's a complicated problem -- set of problems that we're addressing. And I think that it's very clear that the more people that have a chance to think about it and discuss it and hash it over and finally come to a composite plan, the better plan it will be in the end. This is our first real step devoted in focusing on the problem. Before we have never had any information -- we could talk in the abstract all day. Now then, we know something roughly about how many strains are down there, something about the distribution of some of those strains relative to the rest of Mexico, really this is the first time we've been in a position to begin to think about things that I preach. static

Graham: Was it one year ago or two years ago that you were doing a base line survey in the area as a preliminary step in a SWASS test?

Richardson: This is Arriaga?

Whitten: Right It was done in the year 1978. There might be some historical data for what it's worth. See, prior to 1978 through -- I've forgotten exactly when they stopped it but they used to have inspectors in that area -- in other words that was the Area V which was headquarters out there at the airport. In other words they had an area epidemiologist; they had a area supervisor and then they had the livestock inspectors throughout the area. And they were actually bringing in samples so for whatever that would be worth, I think you would have to utilize that information with the knowledge that it may not have always represented an equal amount of the livestock inspector's effort throughout the year but at least there would be some numbers there which might help you now. Probably you would have to get someone in Mexico City to go back through their records and pull that out or it might be you could talk with Dr. Rios down at Tuxtla because he used to be the area epidemiologist at one time, he and Jack Delaney. They may still have those records there.

Richardson: Vic Beal left some summaries of this type of Mexico records with us. We've talked about it. I remember in particular we were focusing on the differences in effort -- sort of an intangible in some ways yet you can make some rough adjustments.

Whitten: That's right, in other words, I know that in that area there the first man who worked with us over there had three different municipios and he would spend a month in each one, so that would mean that maybe one month you would have a real large number and that was probably the month in which he worked there, and then for the next two months when he was expending most of his effort in one of the others -- maybe the numbers went down but there are some numbers there.

Richardson: The patterns might be real but they might be accentuated by him being where the need is the greatest.

Whitten: I hesitated to bring that up, but there are some numbers there.

Richardson: Yes.

Whitten: But I think you would have to realize what all was involved.

Richardson: See, one of the things that kinda highlights the need to get really gonna try to concentrate, it would be nice to get it under the most morphological features instead of karyotypes and enzymes to identify these things is that wherever these numbers exist they still don't tell us what they're comprised of and the different types. One time you might get a peak of one type and another time in the same area a peak of another type. We don't have any seasonal information on these different types but if we had morphology so that you could take the same kinds of specimens coming in for identification or if we had to get adults, trap bait, or whatever, the normal kinds of samples you've been getting, you might be able to get some additional information even though it might not be as accurate or whatever. If it's correlated, it's good enough but it's better than nothing in the end.

.....Static.....

Whitten: Yes, that year that we were over there, the rains actually began about the middle of May and ended about the middle of October because I understand that that was a relatively long rainy season.

.....Static.....

Whitten: May through October rains almost ... and that was basically the way it was

Static begin to go out, that would then rank it as more sensitive than

the one that continued more or less like it was before That's the reason.

Whitten: Well, you'd certainly have to bring it around the way

see the sampling is going to be the limiting factor all the way through, if you

Static here by samples, then there is very little information that we're

going to be able to come back and say what's going on.

Richardson: If you have enough flies static

There's two advantages to having a large population to start with. One is

you can get enough numbers to measure responses and the other is if you're

really gonna try to eradicate, it would be nice to put it under the most

difficult of circumstances. Along that line, one of the things that I think

that we feel fairly strongly and unanimous about is that we ought to start

with a very low density analogous to the lowest ... of the thing

if you don't get a response at all or if you get a response on one that

matches ... or on one of the mismatches ... in density ... see if you can

knock out others in that way, then you might be able to rank density vs.

response. I understand, that up in the area around Salina Creek they have a

Coppedge: ... Static ... it's virtually nothing so I understand ...

Richardson: It's not going to be that clear cut and that's the reason I said

that we hoped we could eradicate. And you can do that -- one is that if

you're getting some suppression of a resistant fly others will be going

out of the picture So, what you're going to have is different proportions

like one going down in proportion and like the other one going up. It may be

going down and absolutely nothing, but not as fast, so you get your lines

and proportions crossing, ... most responses have been in higher proportion.

Going down fast doesn't mean they started lower -- they just collapsed --

they started very low and went out fast ... and ... you ... your release rate ...

then the line should tip even faster til you get vs. the lag time. Of course

after you increase your release rate, then your response would go faster and

some might get quickly and others not much at all. We wonder if they did

respond and begin to go out, that would then rank it as more sensitive than the one that continued more or less like it was before That's the reason, see the sampling is going to be the limiting factor all the way through; if you can't get numbers by samples, then there is very little information that we're going to be able to come back and say what's going on.

Graham: As Knipling pointed out in December, this is going to be very difficult.

Richardson: I think that if there is a limiting factor in any of our paper planning, that's the place that can't be overemphasized. The sampling is going to be critical ... static ... that may eliminate a lot of these questions that tend to be asked on paper that simply can't be effectively answered by any kind of field effort that ... static

Whitten: Up at the northern end of it, in other words, up around Arriaga, it gets, or it was that year extremely dry because you see you go on up north of there, so I understand, that up in the area around Salina Cruz they have a tremendously low annual rainfall; it's virtually nothing so I understand ... I haven't ... but as you work down southward toward Guatemala, it's much more humid throughout the year and it seems like right in that area there, let's say from Arriaga -- well even from Arriaga to Pijijiapan, there is a world of difference even in (what is that about 50 miles, something like that).

Graham: But it's dry enough that all the way to Tapachula they pasture-burn.

Whitten: Yes, that's right.

Graham: Well, it's usually about March or April before they begin burning, isn't it.

Whitten: Sometimes they begin burning in February and I would not --

I would hope that you would not be forced to utilize this time of the year for anything because I don't know what on earth that smoke would do, but it's horrible.

Unknown Voice: Usually by May

Whitten: Well, who knows, but usually yes. I think you could go back and you could look at the records and I think that would help you to decide that on an average year I think could look at how many samples they had -- if they all of a sudden shot up every year during May, that would be ample indication.

Static ... low voices

Whitten: I don't really think that you or that anyone would be able to accurately predict when you're gonna have these times in which it rains every day for about a week, but this usually happens when there is some kind of storm moving through the area. But on the normal days or your average days it's exactly like Hugh has already mentioned then, that usually Mexico or at least in most areas where I've been, mornings are always beautiful and I use the word always to mean most of the time.

Graham: I bet that this weather you saw was when there was a Pacific storm.

Whitten: That's right -- sure.

Low voices: ... open area (Ellison?)

Graham: To go back, you mentioned build-up a while ago. I'm not sure you have to have a population build-up, Will. I think it's when you have

a change in weather that you have increased adult activity more than a matter of building-up through a period of two or three generations.

Averhoff: In other words, May on to ... you have to start

Whitten: Well, if I recall when we were looking at that area, you remember Casarubias gave us all the screwworm samples from the year before and the months of March and the month of April were the lowest numbers, and in May the numbers went up quite a bit ... I've forgotten what they were but I can probably look in my records--in fact I think it's in one of those quarterly reports, but that year, and that was for 1977, and during the year 1978 when we were there, that's how it happened.

Low voices

Whitten: Yes.

Richardson: ... maybe then as a tentative time table we might start, sorta try to get poised and ready to go -- say the first of May and have everything ready and somebody there--you know, monitoring -- if it happened to be an aberrant year -- you know it could be delayed a little bit or possibly even moved up a little bit - more likely - just trying to start on schedule.

Coppedge: Low voice....

Richardson: Will, let me say ... everything's open ... there's nothing Carter can stone here.

Ellison: I'd sure like to release a small collection going into the field test.

Averhoff: But, if, you say, started collections a week before you started releases, you'd still have

Richardson: I'd say a lot of the pattern of collection depends on how good our resolution is by then. For example, if Ray Gagné comes up with some nice, neat morphological features where we can do large numbers relatively easy, then a larger sampling in the field could be handled, but there's no point in making those samples if we can't do anything about it once they come in.

Coppedge: Low voice

Richardson: Right - that sounds like the way to go -- not only do you get a base line going in that way, but you've got your people working in the area, they've got time to go through a dry run of a sort and know what they're doing and you have worked out a lot of the wrinkles. Yeah, I think that's

Coppedge: Low voice

Richardson: Static Sure, and you've got proportion data there too and a different season, and you've got, and a different year for sure, to cross into a season that you've sampled before.

Ellison: Jim, when you say the pasture is burned, do you mean they ignite ... them?

Whitten: Yes sir, they still practice what, or at least what I was told, is Mayan agriculture. In other words, whenever its dry, because they don't

have John Deeres or International Harvesters to get in there, rather than go in there and plow under all that stubble, they remove it by burning and this extends over into the areas where there's heavy brush, grass, and everything. So, you just get smoke--I know that you have never been down there but I know that Will has, I have seen it for, well through the month of March and through the month of April, where you couldn't even see those mountains on the north side there -- just for the smoke.

Ellison: I've seen similar forms of agriculture practiced in Hawaii, where you could not see -- for days at the time.

Graham: This is the milpa system -- it takes out a lot of undesirable vegetation. Also, takes the place of a lot of 2, 4, 5 - T.

Whitten: Well, usually, they do not deliberately burn those areas in which they have improved pastures. Now, that doesn't mean that they don't get burned sometimes ... sometimes they do, but if they have animals and the forage is of value, they don't go out and burn it.

Graham: So far as I know, there is still a lot of Guinea grass and it thrives on burning.

Richardson: Sure, that's the only way you can get it green. I presume they don't have wooden fenceposts.

Whitten: If I might remind you of something else, since most of my experience has been with getting flies ready, if you were going to release flies in May, from right now, you'd have roughly 6 or 7 generations to get

a strain ready and expanded and up to the numbers which you would want and if you were gonna make releases (just a one-time release or if you wanted to make releases for one month and you wanted to do it every week), you'd need to know how many flies you would need and need to have some arrangements made to where you'd have enough in order to go about doing it because that usually involves more effort than what one would originally think. I know from experience we, you know we always shoot to have it done by "X" day but invariably something happens and

Richardson: One advantage to starting off with a low release rate is that one could then increase the supply I think that's some of the kinds of crucial, practical problems. We're in no position to say one way or the other -- we're going to have to take your--I don't know who's going to do it--in fact, when you come right down to that. I mean, we're gonna have to decide who's going to be responsible as well as who's going to or how it's going to be done.

Low voices:

Static:

Richardson: How big an area?

Unidentified voice:

End of side 1 - tape 1.

Tape 1, Side 2

Richardson: One of the other things that was firmly impressed on us by Vic Beal, and we've heard it a number of other times, when we were kinda receptive to pressures and that is this business of swath width - flight width - and also down there it may be even a greater problem if they're not as active, not as mobile as the average from experience. Maybe we can go with one mile flight lanes and even John said he was talking to one of the pilot-type down there about some black box arrangements to keep tabs on where they are rather accurately and all. (Interruption) That's right, it was last night. Maybe they might even alternate one release to the next so that effectively you get a half a mile alternating separation--for dropping.

Static and low voices: ... chase planes ... program before you start out

Richardson: See the idea there is that if one airplane takes--can handle it-- it would be an ideal situation to approach the company for a demonstration and not have to buy the equipment -- get it installed on the planes as a demonstration and thus serve our purposes as accurate control of where they were flying as well as recording where they were flying for any question that would come up at a later time.

Low voice: ...you need to have very good records

Richardson: We were thinking that a one-mile swath would be desirable for a number of features as well as practical and then even they could be telling you know, to shift a little off one way or the other from one time to the next the drop effectively would be essentially half-a-mile swath. See, the idea here is that one of the things here that I don't think we do wish to test -- is swath width effect---what we'd like to have is as uniform a distribution

as possible so that's not a variable later on and we have to come back and sorta grind in some excuses for, and

Coppedge: Low voice

Coppedge: ... static

McInnis: It seems to me that ... static ... one treatment in the whole area ... static

Richardson: There are some other ways that you can test that even in laboratory stage ... several things are coming in the back of my mind here. I think your concern is right--in terms of extrapolation but there is another variable here I think that really hasn't been addressed in any of the discussions that we've been in on and that is the rate of dispersal--natural rates of dispersal--are likely to be much less as you go south and there are a number of reasons why that might be thought to be so. And this hasn't been tested -- this may get to be one of the tests of the future you know, in terms of logistical plans of the program and we find that to get adequate distribution as you move south in the eradication program that the swath widths have to get smaller to do the same job that the wider swath width did farther north. And it seems to me that one thing that we can forego in this particular test--we're primarily testing the genetic match and mismatch--is let's not confound the possibility of distribution of that sort. Now, distribution as a function of genetic differences among strains for their dispersal ability is certainly a valid feature to score the whole system on but this could be measured in terms of activity, maybe even fused to moisture, wind movement, or whatever. These kind of things can be done in laboratory tests to give a pretty good idea of what they will be doing in the field and one doesn't have to then go blind and say that dispersal is

Static and low voices

Richardson: Good point! That settles the issue. I had no idea what the problems were. I think that settles the issue right there.

Coppedge: Low voice

Richardson: And if you're dealing with endemic types--there's good reason to say that natural selection is going to work against their dispersal ability, whereas if you're dealing with widespread types that have to re-invade to get in (so as) to exploit certain resources as they cross the northern part of Mexico -- natural selection in that case might be in the opposite direction for dispersal ability.

Coppedge: In Aldama--two mile swath distance--wind pattern

Richardson: I see, effectively that's overlapping.

Coppedge: You have to overlap anyway. I wouldn't want to ... winds--effect on dropping boxes

Richardson: That sounds like exciting country ... not to be there.

Graham: I was telling Will earlier about stopping at this filling station called La Ventosa and I had to walk about a hundred yards to the filling station and I didn't know whether I was going to make it or not.

Low voices

Graham: It's near the town of Tehuantepec.

Richardson: I think from just what you've said, I'm satisfied with two miles.

Averhoff: I think you're right ... it is ... two planes in there

Richardson: Well then, and the box strip too, see that kinda helps mix it up--what we're really saying is that you've got another source of variation there greater than the swath width so even if you had a more narrow swath width you haven't achieved a thing.

Graham: And you can fly one pattern one time and another pattern the next, right?, which gives you one mile anyway.

Richardson: That's about right

Graham: If your flies live 10 days, you've got your one mile

Richardson: Well, I know in all the discussions with Vic Beal, he was never concerned about two-mile swath widths but he was especially at ten and even sometimes with a five.

Ellison: I think something else too, Dick. The fly that, I take it that you've pretty well decided to drop, is not one of the endemics.

Richardson: That's right, but I mean we still haven't gotten to the question of like Don was saying a while ago, one or two, or one and then a second, or one and two together, or whatever has not yet been decided. I think that I would vote strongly for one and leave somewhere in there being a common type with as wide a distribution over the country as possible. As it also turns out, those are also usually the types that are heavy egg-layers and cause a lot of wound damage, and they are economically probably the most severe of the types we've found anywhere, so for a lot of reasons I think one of which we'd like to make a very high priority and see if we can get rid of it quick and with as much responsiveness as ... you know, that type of fly. There may

be other things that we could choose and another type might be an endemic-- might be an endemic type from another area even, so that never getting into a variety of new questions we can get at As long as we're dealing with one that's going to be a widely distributed type, likely to be a good disperser and all of that, you're right. It lessens the question.

Coppedge: Low voice

Ellison: Right. That's exactly the same conclusion we came to.

Richardson: For the same reason, you don't want the distance between boxes too great.

Voices: low

Ellison: Now, tell me how brave your pilots are. Because I can think of absolutely nothing more dangerous than flying along the windward side of a mountain range, with the Pacific wind blowing you into it.

Richardson: That means they won't go very close.

Ellison: That means if we're not going to drop, if they're very careful they're not going to drop next to that mountain. Well, I would not question the intelligence of any pilot who respected such hazards.

Graham: Yes, they'll fly such an area. They'll drop in the mountains but from greater heights.

Ellison: But the winds coming up over the mountains are not conducive to the boxes going down. They are blowing almost straight up.

Graham: Yes, but those boxes are going to come down.

Ellison: I have seen a parachute gain altitude. likely going to be important, since we don't have any seasonal fluctuation data of the different types, is
 Graham: Yes, but those boxes won't.

... Pause ...

Whitten: Well, what we ended up with there was that from the egg masses that we got from the center of the area, naturally we got the highest rate of egg mass sterility, and as you moved outward and especially along that river this dropped down to the extent that at one of our sheep pens which was up right near the edge, in fact I was worried about it earlier, and we ended up with only getting three egg masses from that one, during the $3\frac{1}{2}$ weeks, after they had begun to make the releases and all three of those hatched, but as you moved down and as you moved more in toward the middle of the area, we were running 70-80-90 percent sterility, depending upon where it was. But there I think its a little bit different there than what it is over in Arriaga in that there are no large rivers that run throughout the year where the flies would be historically moving down those valleys.

Low voices:

Whitten: Yes, oh yes.

Ellison: What was the? and that's the unknown. We may have

Low voices:

Richardson: Is there ... there's an area outside to the south, as I understand, maybe not to the north, that samples, well outside the drop zone could be

Low voices:

Richardson: Let's see, one of the things that's likely going to be important, since we don't have any seasonal fluctuation data of the different types, is to have an outside area sort of serving as control as to what are the proportions undisturbed by the drop--if there are going to be differential proportions, is there a primary measure of differential responses. We've gotta have a good control point from outside and that should be one established ahead of time as it is--as being a typical spot relative to the test area.

Coppedge: Low voice

Richardson: If it's still typical--typical meaning that it still represents what would have happened if you hadn't been releasing sterile flies. If you could make that assumption with some assurance--that'll always be our reference point down there, see. Because, if we're getting normal seasonal shifts in proportion, see, we've got somehow or the other to get that unconfounded with changes due to the release. So, that's a very crucial part of the experimental design.

McInnis: I certainly wouldn't question that a co-area would be desirable and I suppose ... practical ... if the area of the test area had predictable responses on the records year to year.

Richardson: We don't have the types and that's the unknown. We may have absolute numbers but we don't know that A and B are always in the same proportion. They may be going like this. In fact, that's the typical thing from other insect data we have.

McInnis: ... if it were the case ... in terms of total numbers

Richardson: The one thing that, you're right about one thing though Don. You just didn't quite say it. We've talked about it before and that is, if you do monitor the things that are fertile in the release area and they are predominantly the mismatch type, then you do have information about the sensitivity of some types to the released types. That, I think, is also necessary and it would be nice to have the outside control too because what is likely to happen is that you get some absolute diminishing even in types that are resistant in the area of the release, and so it gets harder and harder to pick those up, see, and your sampling is full of duplicates perhaps that is essentially it.

Ellison: Jim, what was the distance between your outlying sheep pen--the first sheep pen--where you say you got 70 percent?

Whitten: Well, it's hard to say but if they flew it the way that we had it drawn, the one we had that was nearest the edge was right up in one corner and it was roughly five miles either way to the area where we were not having flies released. In other words, if this was the area here we'd have flight lanes running this way--we had one right up here and it was five miles down here to the edge and five miles over here to the edge.

Ellison: And you were still getting lots of them?

Whitten: Well, we got three egg masses here and they all hatched.

Ellison: How far away was the first one that you had significant suppression?

Whitten: Well, we had one that was quite a ways back down here. I don't know exactly how far but let's say this was roughly 30 miles here like this, so I don't really know how you'd measure but let's say it was in about like this, so if we'd say this was 5 miles, then this would be roughly 10 - 12 miles,

it was running 60 percent sterility, but it was right on the river, and as dry as it is out there I suspect that the flies if there was any movement, it was up and down the river.

Ellison: So you'd say at least 10 miles.

Whitten: I think you should, I'd think it would be good if you could have that much of a buffer zone. Now I think it would be--I don't think you have to worry about it as much over in Arriaga as it was in that one particular instance because of the geographical conditions -- other voices No, no you don't have to worry about

Richardson: How many sample sites do we have? I mean, what is the distribution and the numbers and what that number _____? one inside and one outside or three inside and one outside or? Well, the more, the merrier, but I'm asking, I figured this was going to be a limiting factor, because they're probably expensive to set up

Graham: You're right, they're expensive and they're a heck of a headache, but we don't want the experiment to come up short of data because we didn't have enough sheep pens.

Richardson: Yeah, well, this is like that two-airplane business or not but

_____?: You can also like a problem.

_____?: Yeah, from natural infestations

Graham: If you can get enough Commission inspectors, right?

_____?: How far are Meadows and Pineda committed on this test?

Coppedge: low voice

Richardson: You can always use some more if somebody makes it happen.

Low voices ... static standard fly release?

Richardson: One of the things I was trying to get a feel for before I really asked another question I am interested in, that is, if you're going to have a control sampling outside the drop area, you need to put what you might consider too heavy a focus by normal standards on that because it's a reference point, you need that bench mark, pretty well known. I don't know if that means two sheep pens out or if it can be done with one, again it may take five, I just don't know if you can react to getting samples, I would say that at least 20 percent of all the samples ought to be from the control area.

Coppedge: low voice the factory area.

Richardson: Yeah, the best of all worlds is that all of these minutiae - details - are unwarranted, but if we don't know I think we got planning for sensitivities that we would consider important but maybe for a larger effect.

Low voice

Richardson: (Has) John Spencer been working in that area over there, has he been static Does that keep him busy?

Coppedge: low voice ... maybe 3 or 4 sheep pens.

Richardson: That doesn't seem like a problem.

Coppedge: If you put one man down there ... 3 or 4 sheep pens.

Graham: If you do that James does it still leave area for a standard fly release?

Coppedge: low voice the release conditions.

Richardson: What's a standard fly release?

Graham: Well, if you were releasing another strain, perhaps the factory strain, in the standard manner.

Richardson: That's more or less what we talked about, but again, you know, we can always change our mind on that as we really haven't hashed it over very carefully.

Coppedge: low voice

Ellison: I think it becomes a little bit of a question as to what you would be testing--not just the factory strain.

Whitten: What is the main goal of this? I wrote down while ago that you (Richardson) said that your main goal was going to be to look into the possibility of a genetic match or a genetic mismatch. Would that be what would be considered as the main or as the primary goal of this research? Or is there a better word for it--I was just trying to write it down so I could interruption

Static

Richardson: Well, that's close Static

Graham: So, really to say what you said in different words, we're trying to find out if there is any reproductive isolation (or reproductive barriers) between these types?

Richardson: Under the release conditions

Graham: Right, under the release conditions.

Richardson: And one of the signs of the mismatch, there are at least two components to the mismatch. It can be a mismatch with something that is in the same area normally with the type that is being released or it can be mismatched with something that is normally not there, that is things that are normally in the same area, the natural selection may be stronger than things that are normally not there. Okay? ... interruption Right. So one of the things that somewhere in here it would be very nice to address the question, I don't know if it's a top priority or a second order priority, but it would be nice to find out, in fact some of Don's stuff may go a long way toward getting at this, is in intentional mismatch, it is something from outside the area that may be better generally across the array of things in another area than something from that specific area if you're trying to take out a whole array, one from that specific area may be very good on its own and not so hot on other things in that area, where outsiders may do very well. This is an idea that Will came up with in one of our bull sessions and I think it has a lot of theoretical merit--I don't know how it fits (the factual situation).

McInnis: Well, from the Sinaloa test, the mismatch seemed to work

Whitten: Well, okay, is this going to be more of a basic type research, or is it more of an applied type?

Richardson: I don't like to split it that way.

Whitten: Well, okay, at one time back in the early history of this, there was mention of being able to present a screwworm fly strain of a known genetic makeup based upon what was known about the native flies -- okay -- that would be more efficient than using what is used now. Okay, so that if this experiment went to investigate that, I would say that it had more of an applied research

application, but if it were just what happens if you use a screwworm fly strain that was, let's say, of the long X type and you are monitoring it, I would say, at least in my own book, that would be more of a basic which might be expanded later on then to be utilized and I was just trying to get a better idea here as to which one of those it is, or if it isn't either one of them, what is it?

Richardson: Well, we want both, frankly.

Ellison: And I think you can get both.

McInnis: Following up on your _____. I had thought that we had discussed this at the meeting in Austin one of the seemed to be to compare a strain which by or several strains ... thus comparing such a strain with the factory strain ... which should work as fast if not ... comparing those two groups then would mean One strain in the area ... is something we hoped ... static ... to avoid duplication of effort ... something novel ... static

Ellison: Well, there are at least 3 things which are very novel. First, the strain that will be released will be of a very--you could call it extremely narrow-genetic base. This was one of the primary questions that was asked (of?) the strain--could such an extremely narrow base be used successfully for release? Okay, that's one of the questions that this is answering, I don't think the Sinoloa test even approached that. The second thing is, these flies that will be released will be of a single genotype; they will be derived from essentially one fertile female, so they have to be of a genotype. We have surveyed the area and we know that there are other genotypes, presently, at least four of them. The question is, how does a narrow-base single genotype fly react when released on many different types. That question can be answered by simply monitoring the response of the flies with respect to the

types _____? (in?) such a release. We are pretty sure that it will work under the type that's being released, if it doesn't we're in trouble (laughter), but one can assess how much one can break down isolating factors by simply saturating .?. released flies. Second? --just a second--.

Richardson: Somewhere I lost him general conversation.

Ellison: I had it organized at the beginning

Richardson: Well, I knew you did, but where did it go?

Ellison: And, I think the control needed, is not what DE-9 would do in the area, because that's certainly irrelevant, we--well--I think that's a duplication of effort, we know that DE-9 will work.

General conversation: density, release rates....

McInnis: Well, I think it needs to be tested.... Otherwise, we will never know what a DE-9 release would do in that same region at the same release rate.

Ellison: What release rate were you using in Sinaloa?

Whitten: Based on what ... told me, over most of the area, it was at 1400 per square mile, over the area where we flew (cough) ... it was 2300, 2400 something like that. Now, how far out that extended, you know, but that's basically what it was.

Ellison: It seems to me that, if you were testing, well

End of tape 1, side 2.

Unidentified Voice: ... convincing characters show-- and I'm not so sure that it's

Ellison: Jim, all you have to

Unidentified Voice: ... have to make that decision ... I don't see that it's that much easier, John, if

Richardson: low voice

Ellison: Well, Don, I think what we're trying to

Richardson: Let's go eat a little barbeque, and talk over barbeque

Averhoff: ... in the ... to eradicate an area ... you can either use one after the other, in tandem, or 2 or 3 or 4 or more ... at the same time as has been done in other areas with the composite fly. ... they may have been down there for quite a while.

McInnis: ... how about in that area ... you'll never know unless you test it.

Averhoff: That's true, except that you have a whole lot of reasons to know that the composite fly is the fly that in a lot of areas of extremely high density, you need

McInnis: All I'm saying is ... it would be nice to have a comparison rather than having to assume

Ellison: Don, what would your opinion be if we could show a complete control of one strain Static

McInnis: All fairly high density ... I would be very impressed

Ellison: Trust me, it is high density in Arriaga.

Unidentified Voice: Arriaga has high density.

General conversation: ... 400 or 500 to start with ... 400 ... start at 250?

Unidentified Voice: Yes.

McInnis: ... what are the possibilities on ... I'd be impressed at 400

Ellison: Okay, would this How much more, how much better would it be than the other strain--what are the cost vs. benefits on

Ellison: Okay, I can, I think I can tell you that the strain we are going to release is probably the same strain that you have most abundant in Sinaloa.

Low voices:

Averhoff: There are two ways you could take it--one is the number of flies ... static now if you can go in say and eliminate that then ... static ... we can go in with the strains ... eliminate third

Low voices:

Ellison: And then follow it up with the second strain that's in there and you can eliminate the second strain ... and then you can take the last three out altogether.

McInnis: Well, is there any argument that having the factory strain to test under static ... is there any question about that? I feel that there's

Richardson: No, I don't have any argument.

Ellison: Don, I think that would be one of the things that could be done as additional tests ... I don't think it has the priority it--that you seem to think it has ... I mean, I think, for instance a control zone outside to make

sure that weather isn't influencing the data is much more important. You know, you can divide Arriaga up so many ways

McInnis: Well, I would rate the test on another strain, the factory strain ... almost as important. I would divide up the experimental area

Ellison: You've got what, 70 miles? With buffer zones of 10 miles, that's 40 miles in buffer zones.

McInnis: two ends

Ellison: Well, two ends, but you've got to buffer one zone in the middle if you're gonna split it.

Richardson: Well, I think

McInnis: I would be, I would always have ... what the other strain would

Richardson: I think

McInnis: I think that would be, I certainly think you should

Low Voice: ... 1000 or 1500then you might always suspect that the wild population was the problem, or let's say that new types had come in which you hadn't anticipated, but that ... if you tested the other strain

Ellison: If that happens, I don't think an outside monitor is going to save the experiment; I think we would declare it, I mean, I think that we could concede defeat.

McInnis: We could do ... good to have it

Ellison: But all of the book-learning, and classes, that I have ever had would argue that that just isn't possible. That is theory.

Graham: Well, for insects in general, but not necessarily the screwworm, John. I think that you didn't study that in the book about the screwworm.

Ellison: You know I have had arguments from numerous people that you cannot take what you find in Drosophila and apply it to screwworm--they told me that you cannot do genitalia, that they are all the same--well, they're not the same, they're just like they are in Drosophila, in cryptic species of Drosophila. It's an exact copy of the sorts of variance that you get. They told me that you couldn't do chromosomes, well, you can do chromosomes, and you get exactly what you do among cryptic species of Drosophila. In other words, everytime somebody tells me the theory that is developed and propagated primarily on Drosophila isn't going to work, I find that they're wrong. And,

Graham: I didn't say it wouldn't work, but I say that you can't apply it to the screwworm without substantiating it

Ellison: You can't assume that it's true, but you can plan experiments accordingly.

Graham: Oh yeah, there we agree.

Ellison: Sure, sure. It's just that we've been so damned lucky in making the right assumptions based on Drosophila, that it's really

Graham: Well, I'm not sure that you have proved that these are the right assumptions, but at least you're convinced that they are.

Ellison: Yeah, well, I'll go down to the wire with anybody who says that the genitalia are all the same. It's just not true. There are differences.

Graham: Sure, morphological variation

Ellison: No, they're strain-specific differences

McInnis: Well, that's right, strains are genetically ... I mean have ... variation ... static ... several voices

Ellison: So far, we haven't been able to get--terribly much data on that but we will have some data on that as soon as we get a wet--if you might--and take it back up to Tuxtla--wet meaning not bone dry.

McInnis: Did I hear correctly that you were able to get on board ... get the Mexicans (airlines?) to go along with this?

Ellison: Yea, Will was. You talk to Will. Will got a letter from somebody that was a real doozie. He presented this ... in the airlines.... I think Meadows fixed him up with a letter, maybe from the Consul.

McInnis: The airlines, they didn't want to originally, is that it?

Ellison: He just assumed that they wouldn't want to and he

Graham: No, it was more than assumption. Hopkins got to San Antonio and they just would not let him on the plane until he left the tanks behind.

McInnis: Well, I've heard of the policy in the U.S.

Ellison: Look, the policy in the U.S. depends on the airline; I got on a United jet baggage compartment, and they They wouldn't put it on the baggage carrier ... but they There's no law against those tanks on airplanes.

Low voices: ...

General conversation: ... the number of people killed each year

Coppedge: Why don't we think this over during the lunch period .. I've

Low voices: not within the 1,000 square miles but looking at labor situations

General conversation about a control area continues

Graham: Does anyone have a map ... I may have one in the pickup

Richardson: You could go ahead and collect what the area as you would the

Averhoff: As I recall, the area from Pijijiapan on down, you might be able to go down there and pick out another 40 mile area or more ... maybe not ... as I recall there was an area right in there that

McInnis: I would consider that if you want to test the factory strain ...

Low voices:

Richardson: That's one of those possible holes ... if you want to do something like that, now's the time ... that option

Richardson: Labor requirement in the field and labor requirement making hay

Ellison: But Will, it doesn't take 200 - 50 samples will be plenty Will.

You're going to have all I can handle

Ellison: I think the lab requirement we haven't been talking about very much

Low conversation: is incredible.

Ellison: But, it's going to be hard to relate it to them anyway. lab work

Ellison: ??: I'll go along with that...I just really, we really need some

Ellison: I think the lab requirement we haven't been talking about very much

Ellison: But we do have to get a baseline....

I'm pretty sure I can identify and separate H-23 type from H-10. I don't have

Low conversation: as the other strains; we need them badly. We need adults

from all these strains as we can get, period -- just anything goes

McInnis: But if you had - what? ... if you had 400 ?

But the other thing we need--we need some help from Gagne--I don't have any

Low conversation: put on him to be expedient about his work, or how much

you can't. If there's a way of getting him to put a high priority on his work--

Richardson: Jim, that's what happens when you put two areas together

and that is the fact that you've got a control but ... and we've got a reference--the fact that's the best of all worlds--the end zone down there would be the pole of all the reference points you might need to work out the comparison of

General conversation about a control area continues

Richardson: You could go ahead and collect that the same as you would the control area too and When you start ... then ... collecting early you could get a survey ... represent the same thing ...

McInnis: I would consider that if you want to test the factory strain ... consider it as a test

Conversation regarding the lab requirement ... low voices.

Richardson: Labor requirement in the field and labor requirement making hay out of stuff you have that comes back at the lab. That's

_____: I think the lab requirement we haven't been talking about very much and the lab requirement is incredible.

_____: John, if you'd get these strains identified, then the lab work

Ellison: That's what I keep telling everybody-- we really, we really need some input from Gagné, we really do. Okay, from the adults that you brought back, I'm pretty sure I can identify and separate M-25 type from M-20. I don't have any adults yet from the other strains; we need them badly. We need adults from as many strains as we can get, period -- just anything goes

But the other thing we need--we need some help from Gagné--I don't know how much pressure you can put on him to be expedient about his work, or how much you can't. If there's a way of getting him to put a high priority on this work--

Richardson: Yes, I know at least one Brazilian interested.

I would really appreciate it if

Richardson: ... or getting someone else to take workloads off

Other voices: ... I really don't know what's necessary ... but I do know he dropped it when he got back; he dropped it and went on to something else. But, we need a field method, and we need it approved by the taxonomists that are in the

Ellison: You're telling me. You've gotta remember I run an interesting

General conversation about the need for taxonomic assistance continues

Richardson: I know that's why I said get him completely out of Washington ... get him out of the Smithsonian where he gets away from all those abstractions ... where he can really focus in on these problems. There is ... well, I think that right now the best we can do is only appreciate a small fraction of our need for him and that's already considerable ... but a good taxonomist right in the middle of what we're into now would go a long way...give us a lot of flexibility down the line of things that now are going to be prohibited because of the problem with identification.

_____: The first requirement would be that he would be interested in

Richardson: Well, that was an assumption. ... if he's not interested, maybe there's somebody else in the world that would be interested -- a taxonomist working with something similar either in Europe or Australia or wherever, that could be brought in for a while.

_____: There's a Brazilian.

Richardson: Yes, I know at least one Brazilian interested.

Graham: Who is this? there are behavioral differences ...?

Richardson: I've forgotten the name _____ was talking about him.

Low voice: Was he the one that worked on _____, the one that was a world authority on _____?

Graham: That would be Lopes then.

Ellison: Yes, that sounds--that rings a bell. I won't swear to it though.

Richardson: Yes, one of the untouched areas of this whole project, that is the one that I think is of central importance ... in

Ellison: Not just for field identification.

Low voice:

Richardson: He hadn't worked in screwworm ... houseflies ... muscids

Graham: If he has worked on muscoid Diptera, he could close the gap pretty fast--there is not that much difference.

Richardson: Well, he has

Ellison: I knew you'd see that it's going to take a taxonomist to get the job done in Mexico. I think sooner or later you're going to have to do away with the genetic test, because it's so damned time consuming--you cannot wait 6 months to find out what all's there.

Richardson: Everything the taxonomist can't see and recognize profitably, then you need genetic tests ... then you go back to the hardware, but first screening should be done visually

McInnis: ... presuming there are behavioral differences ...?

Richardson: Right. I think there are a lot of things that a taxonomist is not going to miss, even if he's just cued in, there's nothing better than the Hawaiian _____ to tell you that but as a first line of examination, a good taxonomist can save you, just tons of wasted effort. That's why a good taxonomist working with genetic analysis, ecological study and that stuff is a very important member of the team.

Graham: When you say genetic, does that include isozymes?

Richardson: It might. That's one (clue?). In this particular instance on the screwworms, it turns out that the chromosomes have been a better guide so far.

Ellison: ... they also have been a more laborious guide than the isozymes.

Richardson: Well, that's understood. Also, it has not yet ruled out working within the chromosome types ... to grapple with that problem, but if we could work with morphologically identified chromosomal types, if we had good morphological features to sort quickly large numbers of flies and not have to do the chromosome analysis to get that same group, then we'd be in a position to do a reasonable isozyme analysis and the level of resolution there is probably higher than chromosome analysis, but it takes numbers, so I think that what we're seeing is different kinds of tools and each has its place and at the present time the one that is in the forefront is chromosome analysis.

Ellison: Dick, each has its place and each multiplies the other's effect.

Richardson: Well, that's true. We use the isozyme analysis as an adjunct to the chromosome analysis where we need it and there are situations where

Low voice:

Ellison: Sure, we can do it. We can collect them, put them in liquid nitrogen and analyze them for quite a while But, what I'm saying is what you need to do, Arriaga is not a place that you particularly care whether its eradicated or not, but the area around Veracruz is, which means that in order to attack a locality in this sort of way, for real, where you really want to send it to zero, you really wouldn't

End of Tape 2 - no recording on side 2.

Low voice:

_____: There's not many taxonomists in the world, in fact. That's another problem, in fact.

Graham: It's not because of a shortage of people who want to work in this area, it's the shortage of funds to support them.

Richardson: Support--and that support starts at our level. We can't recruit people if they're labelled taxonomists, they're not academically acceptable by modern standards, and I think the people who have that opinion are full of jealousy, and that's a fact but the most attitude carries through in research funds and all of that--that's not a healthy field ... we're going to find

Tape 3 - Side 1

Ellison: ... I doubt if I'll (have?) ... (picture form?) ... but I would like to have pinned specimens, and also genitalia, fixed genitalia.

Graham: I can't imagine that there would be any difficulty with that, can you James?

Ellison: Well, so if you ever find a Dr. Gayné who wants to work on it, let me know

Richardson: And incidentally, another thing, maybe, for the record, I think most of the stuff we've been doing, the genitalia and all the rest, by some people's idea, it might have been misinterpreted, is ... going in and taking over taxonomy, but we've been wanting to seduce a taxonomist (laughter) to learn the technique and if anybody feels the least bit reticent to jump in because of _____ stuff they've been doing, then tell them to forget it, that they would be more than welcome.

Low voices:

_____: There's not many taxonomists in the world, in fact. That's another problem, in fact.

Graham: It's not because of a shortage of people who want to work in this area, it's the shortage of funds to support them.

Richardson: Support--and that support starts at our level. We can't recruit people if they're labelled taxonomists, they're not academically acceptable by modern standards, and I think the people who have that opinion are full of baloney, and that's a fact but the same attitude carries through in research funds and all of that--that's not a faddish field ... we're going to find

ourselves in a great shortage of

Ellison: ... low voice ... getting older every year

Coppedge: Getting back to ... (would--did you say?)

Ellison: Okay. Okay, let's call it M-25. I gave him a description, I gave him copies of the larva and I've got pictures here.

Low voices:

Ellison: That established a file in the computer, and everything that's like that goes in the same file. Actually, M-25 ... M-25 is from Sinaloa, but there are, oh ... yeah--it's a type 2 here. Okay, M-25 ranges from Sinaloa clear down to Arriaga, it is a widespread type, it has genitalia identical to AUS D1. By all reasonable criteria, I can't separate them. It is a very distinctive karyotype; it comprises about one quarter to one-third of the flies that Will sent back.....

Low voices ... (McInnis?)

Ellison: ... it is the most prevalent type, it does not comprise the majority .. but we have, one little tidbit just to give you an idea as to its range, one of the flies that (?) we collected west of San Angelo (it's a ranch called Cannings?) that we kinda set off in a group by itself in our survey of '78, has this identical character, so I don't know what the northern limit of its range is, we have picked it up as far north as Culiacan (one sample) except for that one test

Low voices: ... Well, M-25 is not the one that we're going to use probably, we're going to use a stock called IMR-14, which is the same karyotype as M-25.

Graham: You have living material of that?

Ellison: It is a living material stock.

Whitten: ... garbled ... several voices simultaneously.

Ellison: It is from a female, wild-caught, egged in the laboratory, right?
So it is a formal isofemale line

Low voices

Averhoff: Do you understand what the ... I have Type 1, and then I have a stock number, that is the stock that you actually have, and then I have like ... and then there's a file (name? number?) there essentially, so you have IMR-14, F1, see, ... well no, but that's all I've got, all I've got is F1

Low voices continue

Coppedge: ... more than two dosages

Richardson: Parts of it depend on what happens the first time

Richardson: ... that's a ... right there's a time consuming analysis ...
by then it'll be June and you can go down to Tuxtla and wait at Tuxtla ...
low voices

Richardson: No, actually, --I, uh, Lynn can't go, and Mary won't be able to go by then; that means you'll have to send somebody else, maybe Don can plan to go, or somebody, you and one or two of the others should be down there analyzing, ... those larvae ... probably(low voices) ... I wish that I had known.

Ellison: Do you have, do you have one with a generator on it?

Low voice

Ellison: I need 10 amperes at 110 that's fairly constant

Graham: Well, if you set up a trailer there at Tuxtla, then there's no problem, right? You don't need a generator.

Richardson: That's actually what I was thinking. Just in that area--otherwise we've ... got transportation problems ... getting ... back here ... we're going to need somebody on the site

Low voices:

Richardson: How about the possibility of freezing them in the field and sending the tank back by the pilot who drops the flies ... can he land and pick up?

_____: You'd have to bring back pupae or eggs or whatever.

_____: a lot better than up here, I'll tell you

Whitten: Well, I think what he meant was another one of those trailers like

_____: Oh like they got from? Well, they have that one that was always called the night club.

Richardson:

Whitten: Well, that's the name we inherited from when we got it from the Commission. Well, it was the only building there--it set up on a hill there.

Graham: It was just a temporary concrete block building that they threw up-- when the construction people were building the factory, and they used it as a place to set around at night, drink beer, and kill time while waiting to go back to work the next day.

Ellison: Basically, all I need is about a handful of sockets to plug into The one thing that eats you alive on a microscope is vibration

Low voice:

Ellison: If there is a concrete building as opposed to a mobile home well, you know what vibrations are, Don.

Low voices:

Okay, I have set up microscopes aboard ship you can make them work

Low voices:

.... and earthquakes, if you will.

_____: there's room down there in the plant, isn't there?

Graham: That would be ideal for a microscope.

Richardson: Yeah. Now, there's another possibility that may help matters some on this ... some of these can be sorted out electrophoretically maybe even as adults, one of the things that we haven't looked at more than just a smattering is that differences of these types of adults and to me many of these initial identifications might be made by ... and then the chromosome analysis on those can otherwise be done. So, if it turns out that we could make that split, which we'll know by then, we might want to set up one or two plots.

Low voices: ...

Whitten: Well, we don't have anything down there.

Low voices:

We do have a still down there

Richardson: That's the biggest single problem. I know when I set up a lab down at Sao Paulo --- if the water is variable, it can really blow you out of the woods, so to speak.

Whitten: That's right, nobody ever really realizes it until you get in an area where you....

Ellison: If that little Velco still that I have

Richardson: That little Velco still ... when we were working with the Austin city water supply wouldn't do the job

Ellison: The little one?

Richardson: No, that little one didn't ... and I went through all that Kline makes

Ellison: The Big Velco works and the little one doesn't?

Richardson: That's right.

Low voices:

Richardson: Well, there's distilled water and there's distilled water is what I'm saying and the thing is you need to get down there and set it up and check it out; as a last resort, we can haul it from up here but I hope that we don't have to do that.

Ellison: Let me go through some of the arguments if you will, why M-25 and not one of these

Whitten: Who wants to argue?

Ellison: No, no, but I think you need to know some of the discussion that's been rambling around our lab for a couple of

Richardson: wants to argue

Ellison: ... because your sensitivity is loose ... your sensitivity for telling when it's gone You'd have to look through more--more flies that weren't it, to be sure that it's gone, --no

Richardson: Why don't you pick the one that's the most easily identified, genitalia....

Ellison: That's IMR, that's it.

Richardson: low voice

Ellison: I know that it's most easily identified by genitalia.

Richardson: You haven't seen.....?

Ellison: Yeah, I've got three others that I haven't seen yet, ... in the field.

_____: What do you think? (McInnis?)

Richardson: Well, we reserve the right to change our mind

McInnis: low voice

Ellison: Well, first I think Mother Nature's a bitch, so I'm not so sure

that we really have a definite expectation, but let me tell you what I would expect to happen assuming that Mother Nature's going to cooperate with us.

There are living flies living in the same area, and (omit) we picked them all up from the same site, 3 or 4 different times, so they're, you know, they're even living in the same herds. They are not mating together, at least we have not picked up evidence that they are and therefore they will have very likely some isolation--sexual isolation or behavioral isolation, one from the other. Now, since the M-25 type is a rather broadly distributed type, one would expect that the endemic types would probably be very isolated, they've seen this one before, even if they have a relatively narrow niche, the M-25 being more of a generalist, they've probably seen it and they have probably built up a fair amount of isolation, therefore, if you drop M-25 you would expect that low level, keeping the level intentionally low, I expect full control of M-25 type at levels certainly not to exceed 5-800 per square mile. I really would like to see it at 250

McInnis: low voice ... assuming that we had good quality of released flies, ... what would be

Richardson: Basically, you ought to see it at as good as the best you've got

Ellison: For the M-25 type low voice interrupts ... provided in both collections, you throw away everything that isn't M-25. I would expect this to be exceedingly difficult ... low voice again comments

Ellison: Now, with respect to the other flies, certainly at the low levels you wouldn't expect too much effect, because they have seen this fly before, they have been selected for isolation from it, and I'm talking about selection for isolation from it over the past 50,000 years, I'm not talking about recent selection.

Richardson: One proviso--their low levels here may be high levels relative to natural isolating mechanisms

Coppedge: Theoretically, isolated, you're saying that karyotypes similar to IMR-14 is 50 percent of the population ... theoretically. So, initially when you start releasing this IMR-14 your level of sterility should be close to fairly high.

Richardson: In that type.

Coppedge: In that type ... or we'll just say in the whole population ... say 40 percent to 50 percent ... in time ... IMR-14 disappears, your level of sterility should go down.

Richardson: Yes, in fact it ought to disappear.

Richardson: Depending on the strength of isolation ... now you see this is one of those areas where I think we're going to learn qualitatively new things that we really have no sound basis to predict on/... because at some level we can say that there should be no effect on a mismatch ... but we may be way above that level at the lowest thing we'll test, who knows, but we can find out ... that's one of the things

Static - low voices:

Averhoff: (question about natural population levels)

Graham: Well, I've given it a lot of thought, because I wrote about it in the book that Price and I are writing; I have gotten all the opinions that I could. There's very little data as you know, and Coppedge, McInnis and Whitten may all three disagree with me but I concluded in there--I said it is very, very rare for the screwworm to ever exceed 100 individuals per square mile.

Richardson: _____?

Graham: No, total, males and females.

Richardson: That's what I said, _____? , total number.

Graham: Now, there may be rare exceptions when it happens but in real epidemics like when the Gulf Coast tick was so bad over here around Kingsville, etc., they might have had buildups that exceeded that number but they probably dispersed out about as fast as they built up. So, I think that it's probably not going too far to say that 99 percent of the time they will not exceed 100 per square mile.

Richardson: What evidence, what numbers--meager though they be, what--what numbers are there?

Graham: Oh this goes back to a lot of the work in Florida when they were working on island situations, including some Curacao work, and calculations based on hosts available, and there are several different approaches you can make, none of them are exact

Richardson: Do they tend to agree?

Graham: Yeah, they tend to agree, and, I'm really going out on a limb now, but I think that I have concluded in my own mind this is a species that has no trouble existing at levels as low as 10 per square mile or even less

Coppedge: ... low voice ...

General conversation: ...very sparse on the average, this one takes the

Richardson: See, if they're this sparse,/the average, they could be very concentrated but highly localized, now a little bit of habitat selection would

Graham: Well, anyway, I certainly don't think that we release 2000 or 3000 flies because you want that to outnumber the native population. 300 per square mile ... and they get antagonistic above some level in these little areas where they may be congregating ... and disrupt whatever natural isolating mechanism ... based on ... at a level you don't even suspect

Graham: I'm convinced that they have these specialized habitats that you refer to but I'm also convinced that they move from one of these specialized habitats to the other on a very frequent basis.

Richardson: Oh, but I've got an area here and an area here, both of which are habitats for (type) A and they go back and forth but in between there's an area of habitat B and A doesn't stop there but B is over here too and B doesn't go here or here and A doesn't go here or here--that's the kind of thing I'm talking about.

Graham: You're going one step further than I want to right now--but--

Richardson: Well, you see this, this was one of the things that surprised all of us when we found it in the Drosophila. No one expected it. I went into an area that was described as an uniform area, and we found that 5 meters separated, cleanly, habitats of two different kinds and that the uniformity was because they were collected by taking a net and sweeping and everybody crossed these areas, they looked the same to us, until you get down, you know they're different to the flies, and you start looking and see how the flies might _____ and start picking out subtle differences but I'm just throwing this out as a possibility because we do have this sort of experience in the background and if they're very sparse on the average, this now raises the possibility of such a mosaic, you know, I think something we have to hypothesize as a real possibility, not just an off the wall thing.

Graham: Well, anyway, I certainly don't think that we release 2000 per square mile because you need that to outnumber the native population.

Coppedge: low voice ... theoretically low voice continues

Graham: Yeah, and in his early work Knipling theorized that, with sterile male insects of any species, if they were fully competitive, you only needed to outnumber the native, or wild, population by 2 to 1, and then later he raised that to 3 to 1 and up to 4 to 1, but he never did go above 4 to 1, as far as I know.

Averhoff: very low ... kinda what we were talking about ... 250 ... square mile ... talking about a single type ... 350 to 400

Graham: Oh, if you have enough flies

Low voices continue

Averhoff: I was going to say that it probably ties in here, since there are more

Richardson: Maybe. That's probable

Averhoff: Low voice

McInnis: If I were a screwworm, I'd live in Arriaga.

Low voices:

Graham: I wouldn't argue strongly against you on that, but I still think that might be explained on the basis of a build-up in a relatively small area. If you took that county as a whole, and you could calculate the total number by the number of square miles you'd come back down to a 100 or less.

Low voices:

Richardson: Certainly, well certainly, by one piece of information, if you've got a 100 wounds in that area each with a hundred larvae which fall out at about the same time and eclose, but as you were saying, if they disperse rapidly, you may get this tremendous peak, and they're not fertile then-- you've got a while.

Graham: Well, we had an exception to the norm when we had so much Gulf Coast tick, and I've seen others just like Will here, I've seen South Texas Ranchers in the old days go to San Antonio and buy a 1000 yearling steers and castrate, dehorn and brand them all the same day--and turn them loose. Obviously in that pasture you could have more than a hundred per square mile.

Low voice:

Richardson: I don't know about, but we tried that in Drosophila, that's where I developed the bare earth labelling technique, just for that sort of thing, you just make something like dispersing ... activate masses of them ... later on ... dye works fine?

Other voices: ...

Coppedge: You're not taking them out of the environment, you're leaving them there, marking themselves naturally and dispersing on ...?

Richardson: How long does the mark stay?

Coppedge: Probably not more than (several? seven?) weeks.

Ellison: Did you ever, ... it seems to me that your sensitivity? for dispersion ? could ? you could actually recognize eggs in some cases, eggs of females that had fed

Ellison: Not, if you're having a reactor, you don't

McInnis: Well, it seems to me that ... earlier marked release and recapture ... it seems to me We were talking earlier about possibly testing a lower dispersal ... flies in the area ... solve two questions at once ... basically

Coppedge: It's easy when you once get set up ... red dye

Low voices:

Ellison: Will, you were mentioning some time back that there was a problem with the traps, they just weren't catching flies, is this a problem?

Averhoff: ... that was during a rainy period when the only thing that would move during ... the day was females ... just on occasion, not every day, just occasionally, and ... gravid females, and so

Ellison: Okay, so were there other times when the traps worked well?

Averhoff: low voice Yes, there were times old gal full of eggs can only hold them so long and at the same time

Other low voices:

Coppedge: I've found that over

Ellison: Well, then, I think we could come back again to a situation where scientifically we cannot know what we're working with without good, morphological taxonomy. You can feed and recapture, those are going to be females, most of them ... well, and even then you can't for sure assign males to particular types. You really have no idea of whether you're looking

at a single group of very sedentary individuals ... superimposed on a more widely ranging group, or whether you're working with something with certain intermediate between the two that is a homogeneous population

Coppedge: We were working

Ellison: How about just putting a photoelectric counter on the ____? ____?

Coppedge: Low voice

Ellison: That would do it.

Richardson: Lots of raw data, but what do you do with it? In a sense, that's one of the quandries we're in trying to extrapolate from previous experience when we're dealing with a mixture of unknown composition, it really is very difficult to know how far you can carry what's already "known" ... this experience may or may not be very relevant, but that's the best we've got so we're going to do it until we

Coppedge: Low voice

Richardson: And if we learn a lot that wraps up some loose ends we'll still probably want that second shot to do some

Coppedge: I'm going to suggest second round

Richardson: Okay, how about let's do it this way - let us do a draft of it-- we'll try to get off this week--you all revise it as you suggest, and then we'll look at it again. Let's let it cycle, at least once, between us here because there's a lot of things that have been said here today we're going to forget or we're not going to have it in memory quite right ... be a very important provision.

Ellison: Well, if it happens in Drosophila, it'll probably be in the first draft.

McInnis: You guys are going to have to correct that

Coppedge: Low voices ... basics ... objectives

Richardson: just that right there, ... talking about this morning ... one way to say something. You're going to be more sensitive to some of those wordings and all ... we can hack it like you want it.

Averhoff: ... doing a fairly good job.... be more acceptable to the people.

Several low voices:

Richardson: ... that's kinda one of the things I was trying to get one ... things to think about ... want to do them

Whitten: Well, if you do No. 7, please don't tell anybody about it

Ellison: I agree with you on that! If you tell those ranchers you're going to release fertile flies, I don't want to be anywhere in the neighborhood.

Whitten: I don't want to know about it either.

Averhoff: Well, you know that could be something that would be of benefit ... because if you take one kind out ... then you might be

End of Tape 3, Side 1.

Tape 3, Side 2

Richardson: ... there's an ulterior motive that'll come out in just a minute. If Jim's going to be strung out, and Don's going to be strung out, probably everybody else of a senior-sort at Mission's going to be strung out and can't look after the stock-keeping. What's the possibility of getting maybe even an APHIS-person, with experience, in to do the maintenance of the research stocks, not just a standard technician type, but actually somebody that's been handling materials for a long time; is there a possibility of getting anybody? _____: At Mission?

Richardson: Yeah, at Mission. See the reason I'm thinking there, Mission is clearly a more desirable place to have experimental stocks than Tuxtla so we can get at them--and stuff that's handled at Tuxtla ... come out with temporary set-up down there, do the analyses and that's it ... maintain a culture for a variety of reasons ... I'll go ahead and spill the ulterior motive so that you don't have to--you'll know. Yesterday when I was over at A&M, earlier-- and John was talking with Spencer Johnson; he's had a lot of Drosophila experience and some housefly experience on things like dispersal, habitat selection, cuing, movement, ... some other things, a lot of it's on his own, and he was interested in working on the screwworms, at A&M, he could apply for Experiment Station money to pay his bills, but he's going to have to have living material to work with. I told him that if he had irradiated stuff I thought it might be arranged ... as long as ... it came out of cultures ... and obviously he can't get it from Tuxtla, but if we had it at Mission in culture, you guys could send him irradiated stocks for experiments of that sort. So, that's a specific example that I had in mind, there might be other things of the same sort, that even we could benefit by and also if Don or anyone else at Mission is going to be doing experiments, you need a supply--maybe a sizeable supply of certain types, of things that are

dependable, of dependable composition, a supply where contamination problems are not of concern, and that's a full-time job is what I'm saying, you need somebody assigned to that and that is their sole duty, and they know that, and are competent to handle.

Whitten: I'm not really sure that I understand yet, in other words, could we accept an individual, who would be free to us, (interruption) ...

Richardson: ... assuming that as usual, you're going to be short of money ... that you can't hire somebody.

Whitten: ... could we accept him in our rearing facility so that his first priority would be to rear the insects that were required for whatever this ... to run a test with.

Other voice: ... whatever you know how

Whitten: I will say that I could accept him. I don't know about who I'll ...

Richardson: Well, actually, I guess, I assumed that since we're accepting ... it's a real possibility. Because the assumption I was making there is that you guys ... those of you that have experience are obviously going to be right in the middle of all of these other things that are going on, and not going to have the time to devote to that kind of thing; is that really of crucial importance

Whitten: I think it would all depend as to what all is going to be involved, like lately, or at least, within this last year, we have been, we have had more insects in there, more different lines than we ever have had before to my knowledge, and frankly, we really have more than we ought to have, but

whether or not any of this other could be worked around that, like there will be times, and I hope soon, that we won't have so many lines, and uh,

Richardson: Well, if we just worked with Arriaga, you've got five right there, in some cases, in some experiments, you need a fairly good supply of material, of a fairly reasonable number you know (other voices) ... that's not the kind of thing that you (other voices) ... oh yeah, I was ? five types, oh yeah, in terms of separate cultures, 15 - 20

Whitten: I don't know/^{how} Hugh or James or anybody else feels on this. I would rather that if we had something like that, that it be set up, let's say for a three-month interval, say for instance, maybe like from June through August, let's say, so that we could plan our rearing knowing that we were going to be expected to do more during that time. Whenever it happened to be, I'm not-- I'm just using that as an example ... then at the end of that time, we would have that out of the way and then go on about our business but to do it over an extended time-period, I think that it might work out as well as what we would hope for.

Richardson: Well, certainly, 3 months, or 6 months, would be long enough to do quite a bit of work and that might really be quite feasible, uh, if everybody coordinates their plans so that--we're going to have to do it, all agree on that, and make ... all of that available.

Whitten: And, this would be part of this Arriaga experiment?

Richardson: Yeah, this might be a good excuse for it too, because see one of the things that I was thinking about from our side if we're going to do an electronic analyses of the different (livestock?) ... you've got to have your cultures and you've got to be able to sample them in numbers that are convenient

to work with, this may have them staggered over the same cultures, at different stages; same life, different stages; if somebody else is doing other kinds of behavior work, if John's going to be comparing mating behavior, for example, if Spencer had the support, he could be doing other kinds of studies, these things could be concentrated, I think and made to work, under those limitations that we talked about, certainly a lot of ... but frankly I feel very handicapped from the ... side in ... by not having, and not having a lot of flies of the right sort, you can make test crosses with and have developmental differences or what--even though I'm not interested in the bigger picture about those differences, that where you get a feel, as you know, for the kinds of differences, that you're likely to see when you start looking at wild stuff, and if we could have that sort of arrangement, set up at Mission, we're less likely to get trapped with an unknown that we could have otherwise known, and make a false interpretation, and a lot of these experiments would be helpful in interpreting the overall significance and extrapolating to other areas what we learn down at Arriaga.

Ellison: In that regard, I'd like to jump on Don for a minute. We need to get some mating experiments on these ... cross made, ... well, whatever you think.

Am I correct that Chris is going to make the specific crosses that we asked for?

Chris Hoffman? Or, is it Hopkins?

Averhoff: We need to check that out

Richardson: What's Hopkins going to be doing?

Averhoff: Low voice

Graham: I just mentioned to Will this morning that just before Don left,

Don Hopkins left Kerrville to go back, he just casually mentioned that he

thought he was going to be transferred to Hermosillo--and I said, oh, and let it go at that.

Richardson: That's not good news.

Graham: He may go back to Tuxtla and hear an entirely different story.

Low voices:

Graham: Yeah, and it's to support this new regional organization that APHIS has come up with, too.

Whitten: I hope it's also to support the eradication

Richardson: Well, now see the other side--talking about the coordinating of these kinds of availability of these materials these things also.

Averhoff: low voice

(Discussion of large cages and their use in behavior studies continues for several minutes.)

Graham: That would be a tremendous breakthrough if you could get what you're aiming at there but, I'm like James, I've seen efforts made for 40 years and nobody's made a breakthrough yet ... maybe you're the man that will

Averhoff: I don't know, but I sat on one paper for 3 or 4 years just because of _____ ? ... couldn't find ... so, I'm not saying I can with screwworms, but I'm willing to try.

Coppedge: Low voice

(Exchange of conversation about mating behavior continues between Averhoff, Coppedge, and Richardson) ...

Graham: And they're not dependent on any one species of plant either -- it might be one plant in one area, another in another, one time of the year, and another time of the year.

Richardson: The thing is, I guess, as fundamental as that is, they're so thinly spread, or at least its such a rare chance of finding them naturally, that the best I think we could do is hope in a situation with some control that Will can pick up some cues, initial indications at least, at a reasonable try -- now, it may turn out that he never gets what one considers normal behavior, but from any modification of their behavior he can achieve, it might then be a suggestion for Mackley or somebody else of where to go, and eventually by talking about it ... and it'll strike a chord with some of the people working in the field, that may lead them to that needle in the haystack.

Graham: I would say that if Will can get them while confined to feed as they do in nature, or mate as they do in nature, or oviposit as they do in nature, it will be a big breakthrough -- anyone of the three.

Averhoff: ... that gives me lots of leeway ... not, I wouldn't say in a free ? test

Graham: ... cause I've looked at a lot of them in the corner of the cage and said, "why you stupid so and so, why do you want to hang up there and die for"?

Averhoff: The only, the only thing

Graham: And they would move around from place to place, they'd go drink water, ... or

Richardson: It's a good thing you're going to be down there. You know, when I found out that you were fixing to go, I think that with you and him close together in that sort of thing ... that will be a good ... situation.

Graham: Well, I hope so. The most progress I have seen in the past was about 10 years ago down in Veracruz, an entomologist named Andrew Roth was transferred to screwworms, and he was down there--he built a fairly large cage, about 12 or 15 feet tall and about 40 feet in diameter I guess, with no corners, at least no square corners, and lined with tobacco cloth, and, he had the most encouraging results I have ever seen, but a wind came through and tore the cage down and he retired soon afterward, and that was the end of that

McInnis: Low voice

General conversation in low voices ... for several minutes.

Richardson: That's what I was thinking about--if they didn't want to set it up down there at first, you could get started on it in Austin in the supply shop--or machine shop right there--you can make it, throw it out--modify it or whatever ... was ... convenient ... get it workable

Ellison: Which brings me to another point, Don, what are you going to be doing while we're running the test down in Arriaga; are you going to be available with your microscope?

Richardson: I noticed he was awfully quiet while ago.

Ellison: Yeah, he really quiets down doesn't he?

McInnis: Well, it all depends I guess

Ellison: Please be advised that you'd be very much appreciated.

(General conversation in low voices for several minutes.)

Ellison: If you think that's bad, what about the scope--that's 30 kilobucks. Is there anyway to get the State Department to insure it, or to at least insure me getting it back? How do you handle something like transportation ... voice fades ... Well, no its going to stay with me

Coppedge: ... discusses import permits and their handling by the Commission

Graham: Sometimes you need to use a little camouflage.

Ellison: It's hard to camouflage this one

(General discussion follows on the value of the microscope, its replacement cost, etc.)

Richardson: Is there any way ... get some of these cultures going where they're totally enclosed--such as in a bottle? Instead of out in those open pans and stuff?

Whitten: You mean during the larval stage?

Richardson: Well, closed at all stages, really, but maybe in different containers, or containers within containers, ... what I'm really thinking about is, you have to rely on other people, and also have more, to ... take a fresh look at how you do it in the first place, for example in the Hawaiian Drosophila

where the larvae crawl out and pupate in the soil they oviposit in a vial of food, and the larvae grow in there, but actually there's two kinds of food (inside), they oviposit on a band of food on the side of the vial, that's one kind, and the larvae grow in another kind of food that's in the bottom of the vial, then when they get to the third instar and ready for pupation, you take a whole bundle of these vials, pull all the plugs out and put them down in a

big jar like this with sand in it, they crawl out of those vials, go down into the sand and pupate, then later on when the adults come out, you aspirate the adults out with a vacuum pump, so it may take some manipulation that way, containers to containers, but at all times, the containers are completely physically excluded from everything on the outside, and the larvae can't get in or out, and the whole thing is contained--that type of operation.

Whitten: Well, that could be done except for the larval media ... I don't think you could put enough media in there in order for the larvae to completely develop on, plus you'd also have to maintain this at 98°.

Graham: You would probably have to have some kind of ventilation

Whitten: Oh yeah, because you see the ammonia from that

Richardson: ... from all that protein

Whitten: Yeah, it's something horrible That isn't to say that it couldn't be done, but it wouldn't be easy ... screwworm larvae are not hard to rear, but they require a lot of attention, in other words, I would rather rear them than to rear Heliothis, or boll weevil, or anything like that where you have so many contaminants, and by that I mean the different yeasts and molds, and all of that gobbledygook because we don't have to worry with any of that ... but I don't know, I would say that if there were dire need to ... it might ... but I do know that earlier there was a guy--what was his name-- Ray Moore--was trying to work on a--some type of a--well, one of the reasons he was down there--he may have been there when you were

Graham: Uh huh.

Whitten: ... because Bushland or somebody wanted to see what it was that you had to feed the larvae in order to get a nice, blue-colored adult, right? -- something like that, so anyway ...

Graham: He is an insect nutritionist.

Whitten: ... Ray had these elaborate larval feeding systems, so I understand, where he dripped blood, or some mixture, or some blood-solution on the end of the larval rearing vat and supposedly all of the spent mixture drifted out on the other end and all of this--I never did see any of them but that was how I heard them described ... but the larval stages, they're so much like little babies, you have

Richardson: What I was thinking about while ago was building style--if he could sketch out what you think might be an apparatus that would have all of the necessary ingredients, but entirely enclosed even though you may have to add stuff or take out stuff at various times, but that culture would be entirely contained... sketch it out and let us have one or more constructed for a try

Whitten: What would be your reason for wanting to have one like that?

Richardson: For one thing it would help insure absence of contamination, especially, it might also mean that you could handle more with that same assurance of pure cultures

Graham: You're talking about small numbers?

Richardson: Small numbers, right.

Whitten: I would say about the main thing is ... all you could handle

Richardson: Voice fades....

Graham: I wonder how it would work if you'd put ... your larval media up in cylinders and put them in one cylinder until they're one or two days old, then add another cylinder here, let them move on down into B, and just keep on adding cylinders until full-grown larvae crawled off into the last (clean) cylinder.

Whitten: That might work but they'd almost have to be open

Graham: Yeah, you'd have to open them when you joined the two cylinders.

Whitten: But I mean you'd have to have some kind of ventilation

Graham: Yeah, you'd have to provide ... or forced air.

Richardson: But even if it were a forced air set-up with a little diaphragm top or something because actually, you see, if you needed that, actually what you would do is have a stockroom set up for this thing with air jets around with a filtered air supply

Whitten: Oh, I'm sure it could be done--I don't really understand you fully yet. In other words, you would want the adult to emerge within this thing ... right or wrong?

Richardson: Well, no, you see, that's why I was using the Drosophila as an example. Actually, the adults emerge in a separate container than the part that the larval vials are in, but they just as well be taken out after they crawl out and pupate

Whitten: Then you would just want them to be isolated in the larval stage, and the larval fall-out stage

Richardson: Yeah, when they're egging, when they're larval, until they pupate, and then in a more convenient way, you know, when the adults are coming out, I would think, but such that there's an impenetrable physical barrier between these at all stages where they could get contaminated ... and ... if it's small numbers this way then, you get an idea developing, then it might be replicated and that way you'd have many lines, or more of the same lines if you needed numbers. But have it completely enclosed. If somebody's got some ideas, if you or anybody else has some ideas, we can get it constructed, see very easily, to try

(Two voices:) Exchange of conversation concerning rearing of special strains continues.

Whitten: Basically, that's what they have up at Fargo ... they rear theirs on a relatively small scale, and they have individual ? ... have a vat there (two voices) ... it is heated with some kind of electrical heat from below it; they do not use water the way we do and the larvae are all right here in this area, so it may be 3 or 4 feet over here to the next hood so each one of those hoods is like a small island, and then that way whoever it is that is maintaining those has access to the media and they can add media and remove the old media and when the larvae have completed their larval stage, they move out of the media and there is sort of a little drop area just like we have except theirs' extends all the way around and there's a hole right over here (gesturing with hands) and then the larvae all ... make it to that hole and they just drop down into ... I don't know what they have down there, maybe a jar or something like that could be set up, but that would in essence, satisfy what you were asking for

Richardson: Sounds like it would, it sounds more elaborate than what would probably be necessary

Whitten: Yeah, maybe ... I would say that it would all depend on how many you wanted at a time, but, in other words, each one of theirs is like a small island, for the larval stage

Richardson: Well, yeah, well when they're not very conspicuous, of course, is when they slip up on us; in fact, eggging and the early larval stages are the most critical but also it would be reassuring to know that there was a physical restraint against being mixed ... static

(General conversation about some stereograms of screwworm phallosomes.)

Richardson: ... John brought some stereoviewers over here in the little black box; he had the shop copy from his Dad who used to do aerial photographs ... light box ... use it on the gels, see ... pretty pictures You can see some of these genitalia in three dimensions, that was the thing, and some of those structures we had a chance to show Ray, that he was concerned about earlier when looking at those genitalia, there was a single piece of chitin, that while it's flexible, its not easily distorted and then lots of other neat things--when Ray was in Austin we spent a lot of time trying to figure out how, he had some drawings showing how those different structures affect one another, that's a really complex set of levers, to make that penis work, and in some of those, you can begin to appreciate when it's three-dimensional far more than you can otherwise. The other thing we've got

(General conversation about the terminal and the stereoview pictures continue several minutes. People are moving about and there are various conversations going on.)

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